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ORIGINAL ARTICLE

Can dual energy X-ray absorptiometry provide a valid assessment of changes in thigh muscle mass with strength training in older adults?

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Objective: To determine how dual-energy X-ray absorptiometry (DXA) compares to computed tomography (CT) for measuring changes in total thigh skeletal muscle (SM) mass with strength training (ST) in older adults.
Subjects: Fifty previously sedentary, relatively healthy older men ($n=23$, 60 (s.d. $=7.5$) years) and women ($n=27$, 60 (s.d. $=9.3$) years).
Results: Results indicate that there was a significant increase in thigh SM mass with ST measured by both CT ($3.9\pm0.4\%$) and DXA ($2.9\pm0.6\%$) methods (both $P<0.001$), and there was not a significant difference in percent change between the two methods, although there was a substantial absolute difference (~ 2 kg) at baseline between the two methods. Although Bland–Altman plots indicate overall agreement between the percent thigh SM mass changes of DXA vs CT methods, the 3.4% error associated with DXA was greater than the thigh SM mass change from DXA. However, the CT measured change in thigh SM mass was greater than its error (0.6%).
Conclusions: DXA overestimates baseline and after ST thigh SM mass, and may not be able to detect small changes in thigh SM mass with ST due to its higher error. Although DXA has certain advantages that warrant is used in epidemiologic and intervention studies, improvements to DXA are needed for the accurate assessment of small changes in thigh SM mass.
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Introduction

Accurate measurement of skeletal muscle (SM) mass is critical for determining the effectiveness of exercise interventions (Prior *et al.*, 1997). The most accurate methods of measuring SM mass *in vivo* are multislice computed tomography (CT) and magnetic resonance imaging (MRI) (Heymsfield *et al.*, 1997). However, the limitations of these reference methods, including expense, equipment availability and excessive radiation exposure (for CT), often make their use impractical for large research studies (Kim *et al.*, 2002).

Dual-energy X-ray absorptiometry (DXA) is used extensively to estimate SM mass changes with various interventions (Starling *et al.*, 1999; Newman *et al.*, 2005; Raguso *et al.*, 2005). Additionally, DXA is more affordable than CT or MRI, is easier to operate and uses much less radiation than CT (Heymsfield *et al.*, 1997). Moreover, DXA-estimated appendicular SM (aSM) mass correlates with aSM mass measured by MRI (Shih *et al.*, 2000; Kim *et al.*, 2002) and multi-slice CT (Visser *et al.*, 1999; Levine *et al.*, 2000). However, other studies questioned the validity of DXA for accurately measuring aSM mass (Nelson *et al.*, 1996; Tylavsky *et al.*, 2003a), possibly due to error in determining fat-free mass (FFM) over bone (Schoeller *et al.*, 2005). However, only one study examined the ability of DXA to detect changes in aSM mass with strength training (ST) in older adults (Nelson *et al.*, 1996). That study used older, pencil beam DXA and used single-slice CT for measuring the area of the mid-thigh,

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which is not the most accurate method for estimating total thigh SM mass (Tracy *et al*., 2003). Accurately assessing SM mass changes with ST is important, because ST is the preferred sarcopenia intervention because of the lower number of side effects compared to pharmacological interventions (Blackman *et al*., 2002). To accurately quantify total thigh SM mass with CT, a multislice protocol is needed, as using a single slice is inadequate for estimating whole muscle volume (MV) of the thigh (Tracy *et al*., 2003). To our knowledge, there are no reports on the relationship between multislice CT and DXA for measuring changes in total thigh SM mass with ST in older adults.

The purpose of this investigation was to determine if the change in DXA-derived thigh SM mass is a valid measure of the changes in SM mass with ST when compared to thigh SM mass changes measured by multislice CT. We hypothesized that DXA-estimated changes in thigh SM mass would be positively correlated with CT-measured thigh SM mass, and that there would be agreement between the two methods despite the likelihood of an overall difference in the absolute mass of thigh SM as measured by these two methods.

Subjects and methods

Subjects

Fifty previously inactive, relatively healthy men ($n=23$) and women ($n=27$) between 50 and 83 years of age volunteered to participate in this study. All subjects underwent a phone-screening interview, received medical clearance from their primary care physician and completed a detailed medical history before study participation. All were non-smokers, free of significant diseases or disorders that would affect their ability to safely perform ST. Subjects already taking medications for at least 3 weeks before the start of the study were permitted into the study as long as there were no medication changes during the study. After all procedures were explained, subjects read and signed a written consent form, which was approved by the Institutional Review Board of the University of Maryland, College Park. Subjects were reminded throughout the study not to alter their physical activity levels or dietary habits during the investigation, and body weight was measured weekly.

Body composition assessment

Body composition was estimated by fan-beam DXA (model QDR 4500A, Hologic, Waltham, MA, USA; software version 8.21) using previously described methods (Delmonico *et al*., 2005). Coefficients of variation (CVs) for all DXA estimates of body composition were calculated from three consecutive, repeated scans of 10 subjects (not part of the current investigation) with repositioning. The CV was 0.6% for FFM and 1.0% for % fat. The scanner was calibrated daily against a spine calibration block and step phantom block supplied by the manufacturer. Additionally, a whole body phantom was scanned weekly to assess any machine drift over time. Body weight was determined to the nearest 0.1 kg with subjects dressed in medical scrubs, and height was measured to the nearest 0.1 cm using a stadiometer (Harpenden, Holtain, Wales, UK).

DXA thigh region of interest assessment

The thigh region of interest (ROI) measured by DXA was used to determine the amount of FFM in the thigh, excluding bone, using previously described methods (Visser *et al*., 1999). Briefly, the upper limit of the region was defined as the lowest point of the ischial tuberosity. The lower limit was defined as the superior border of the patella. The pubic symphysis and the most lateral aspect of the thigh were used as the medial and lateral limits, respectively. One technician performed all of the analyses on the baseline and after ST scans. The CV for leg FFM was 2.2% based on the three repeated scans of the 10 subjects described above and was 3.4% for the untrained leg for the subjects in the current investigation when measured at baseline and at the end of ST.

CT MV assessment

To quantify thigh MV, multislice CT imaging of the trained and untrained thighs was performed (GE Lightspeed Qxi, General Electric, Milwaukee, WI, USA) at baseline and during the last week of the ST program using previously described methods (Delmonico *et al*., 2005). Section thickness was fixed at 10 mm, with 40 mm separating each section, based on previous work in our laboratory by Tracy *et al*. (2003). Measurements of MV in the untrained leg served as a control for seasonal, methodological and biological variation of MV, by comparing the changes in the control leg to the training-induced changes in the trained leg. The CV for muscle mass was 0.6% in the untrained leg when measured at baseline and at the end of the training period.

One technician performed analyses of all images for each subject using Medical Image Processing, Analysis, and Visualization (MIPAV) software (NIH, Bethesda, MD, USA). For each axial section, the cross-sectional area (CSA) of the thigh muscle group, excluding bone, was outlined. Thigh MV was calculated using a previously published Hounsfield unit range (0–100) for muscle mass (Kelley *et al*., 1991) excluding all other tissues (for example subfascial fat, bone, and other tissues) that are not in this range. The same number of sections proximal from the patella was measured for a particular subject before and after training, to ensure within subject measurement replication. The technician was blinded to subject identification and date of scans. Repeated measurement CV was calculated for the technician based on repeated measures of selected axial sections of one subject on two separate days. This resulted in a CV of 1.1%. MV was calculated using the truncated cone formula as reported by Tracy *et al*. (2003) and described by Ross *et al*. (1996). To convert MV to SM mass, it was assumed that muscle density was 1.04, which allowed for comparison with thigh FFM (kg) as measured by DXA (Levine *et al*., 2000).

Muscular strength

One-repetition maximum (1-RM) strength tests were assessed for the knee extensors before and after the ST program using air-powered knee extension machines (Keiser Co. Inc., Fresno, CA, USA) and standardized procedures as we described previously (Delmonico *et al*., 2005; Kostek *et al*., 2005).

Training program

The ST program was performed on Keiser A-300 air-powered leg extension machines and consisted of unilateral (one-legged) ST of the knee extensors of the right leg, three times per week, for ~10 weeks using methods we described previously (Delmonico *et al*., 2005; Kostek *et al*., 2005). The untrained control leg was kept in a relaxed position during all ST sessions.

Data analysis

Statistical analyses were performed using SAS (SAS version 8.1, SAS institute Inc., Cary, NC, USA) and MedCalc (version 8.1.1.0, Mariakerke, Belgium) software. Paired *t*-tests were used to determine if ST had an effect on physical characteristics, strength (1-RM) and thigh SM mass in the trained and untrained legs. Scatter plots and Pearson product–moment correlation coefficients were used to assess the relationship between DXA-measured to CT-measured thigh SM mass at baseline and after training along with changes in SM mass with ST. Linear regression models were used to test if there was a sex by measurement method interaction, but none were noted, so analyses were not stratified by sex. Additionally, to compare two measurements techniques (CT and DXA) Bland and Altman (1986) plots were employed whereby the differences in techniques are plotted against the averages of the two techniques.

Results

Subject characteristics are shown in Table 1. Both men and women increased their 1-RM significantly with ST ($P<0.001$). There were no other significant changes in any of the physical characteristics shown in Table 1 for men or women with ST.

Table 2 shows a significant increase in thigh SM mass as measured by both CT ($3.9 \pm 0.4\%$) and DXA ($2.9 \pm 0.6\%$) methods with ST in the entire cohort (both $P<0.001$). There were significant increases in 1-RM of both the trained and untrained legs ($P<0.001$), which was expected due to the well-documented cross-education effect in the untrained leg.

Table 1 Physical characteristics at baseline and after ST in men and women

	Men		*Women*	
	Baseline	*After ST*	*Baseline*	*After ST*
N	23	—	27	—
Age (years)	60 (7.5)	—	62 (9.3)	—
Height (cm)	175.0 (5.9)	—	161.3 (6.3)	—
Weight (kg)	87.1 (14.9)	87.5 (14.4)	72.8 (13.2)	72.7 (13.4)
BMI (kg/m^2)	28.5 (5.1)	28.6 (5.0)	27.9 (4.4)	27.9 (4.5)
Body fat (%)	27.7 (5.7)	27.6 (5.3)	39.0 (5.2)	38.7 (5.1)
Fat-free mass (kg)	62.4 (8.0)	62.9 (7.7)	43.9 (5.9)	44.1 (6.6)
1-RM (N)	340 (82)	418 (98)*	178 (67)	222 (71)*

Abbreviations: BMI, body mass index; kg, kilograms; N, newtons; 1-RM, knee extension one-repetition maximum; ST, strength training.
Values are means (s.d.).
Data presented are for all subjects with baseline and after ST measurements.
*Significantly greater than baseline ($P<0.001$).

Table 2 Change in 1-RM strength, baseline and change in thigh skeletal muscle mass with ST using CT and DXA measurements in the trained and untrained legs ($n=50$)

	Trained leg	*Untrained leg*
1-RM Change (N)	65 ± 6*,†	25 ± 6*
Baseline CT muscle mass (kg)	3.264 ± 0.135	3.192 ± 0.133
After ST CT muscle mass (kg)	3.390 ± 0.141*	3.206 ± 0.134
CT muscle mass change with ST (kg)	0.126 ± 0.015*,†	0.014 ± 0.014
Baseline DXA muscle mass (kg)[a]	5.096 ± 0.173	5.131 ± 0.177
After ST DXA muscle mass (kg)[a]	5.241 ± 0.172*	5.143 ± 0.176
DXA FFM change with ST (kg)[a]	0.145 ± 0.032*,†	0.012 ± 0.026

Abbreviations: CT, computed tomography; DXA, dual energy X-ray absorptiometry; FFM, fat-free mass; kg, kilograms; N, newtons; 1-RM, knee extension repetition maximum; ST, strength training.
Values are means ± s.e.m.*Significant change with ST ($P<0.001$).
†Significantly greater change than the untrained leg with ST ($P<0.001$).
[a]FFM measurements exclude bone.

However, the increases in thigh SM mass (from both CT and DXA) and 1-RM in the trained leg were significantly greater than the change or drift in the untrained leg ($P<0.001$). There were no significant percent change differences in thigh SM mass between men and women using either the CT or DXA methods. Additionally, Figure 1 shows that there was no difference in the percent thigh SM mass change in either the trained or untrained legs with ST when CT and DXA methods were compared. There was a strong positive and highly significant correlation between CT- vs DXA-derived thigh SM mass at baseline ($r=0.88$, $P<0.001$) and after ST ($r=0.90$, $P<0.001$). However, there was only a moderately positive correlation between the percent change in DXA- vs CT-derived thigh SM mass with ST in the trained leg ($r=0.526$, $P<0.001$). Although the slope of the percent muscle mass change measured by CT vs DXA was not significantly different from the identity line (global test $P=0.09$), the data show that there was a ~0.25% underestimation in muscle mass by DXA for every 1% increase in muscle mass as measured by CT (Figure 2). Additionally, hypertrophy was observed in seven subjects using CT with a corresponding decrease in thigh SM mass using DXA.

Figure 3 shows that the percent change between CT and DXA methods were not significantly different, with a mean difference between the methods of 0.89% (95% confidence interval (CI) = −0.151 to 1.940). However, a moderate negative relationship ($r = -0.395$, $P = 0.004$) was observed for percent thigh SM mass difference between the methods vs the mean of the two methods. This indicates some proportional error between the two methods that was shown in Figure 2. Moreover, Figure 4 shows that there was no difference in percent muscle mass change between the CT and DXA methods in the untrained leg (mean difference = 0.13%; 95% CI = −0.817 to 1.069). The limits of agreement for the trained leg and untrained leg from DXA and CT methods for % change in thigh SM mass with ST are −6.3 and 8.1% and −6.4 and 6.6%, respectively. Bland–Altman plots using absolute values at baseline (Figure 5) and after training (Figure 6) displayed the expected systematic

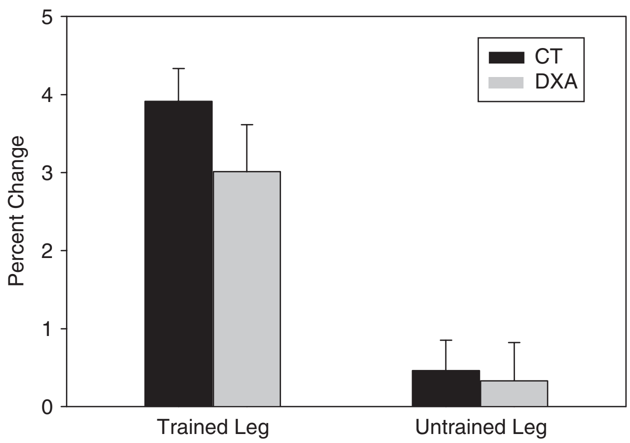

Figure 1 Comparison of percent changes in thigh muscle with strength training (ST) in men and women ($n = 50$) in the trained and untrained legs using computed tomography (CT) and dual energy X-ray absorptiometry (DXA). There were no significant differences in the change or drift in thigh muscle mass between CT and DXA methods with ST. Values are means ± s.e.m.

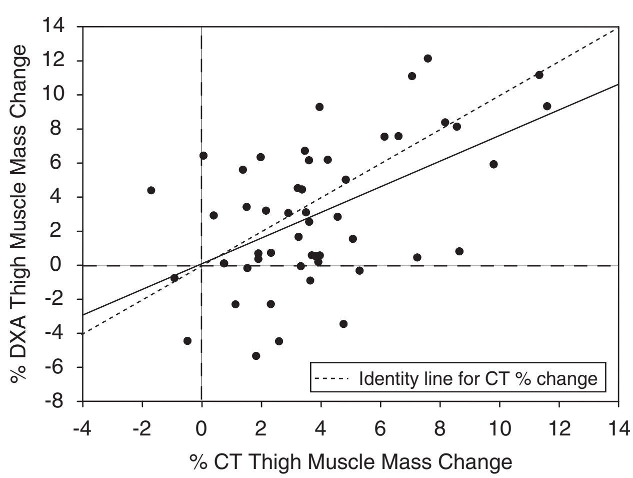

Figure 2 Regression of percent thigh muscle mass change with strength training (ST) using computed tomography (CT) against dual energy X-ray absorptiometry (DXA) methods in older men and women ($n = 50$). Dotted line is line of identity (regression slope = 1; regression intercept = 0). CT and DXA methods were moderately correlated ($r = 0.526$, $P < 0.001$; 95% CI = 0.291–0.702). CI, confidence interval.

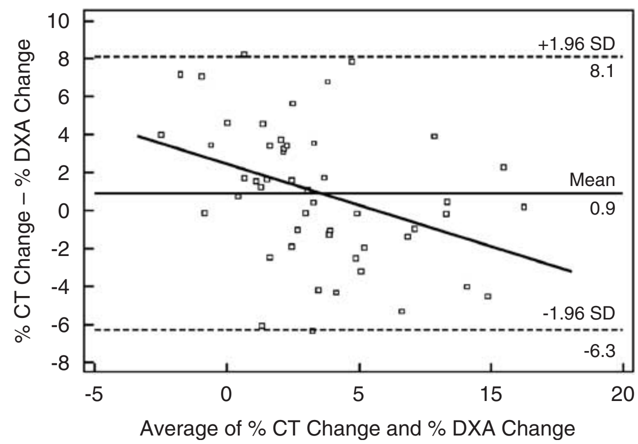

Figure 3 Bland–Altman plot of agreement between computed tomography (CT) and dual energy X-ray absorptiometry (DXA) methods for measuring percent muscle mass change in the trained leg with strength training (ST) in older men and women ($n = 50$). CT and DXA methods were in agreement (mean difference = 0.89; 95% CI = −0.151 to 1.940). There was moderate negative relationship ($r = -0.395$, $P = 0.004$) for the difference between the two methods vs the mean of the two methods for percent thigh muscle mass change. CI, confidence interval.

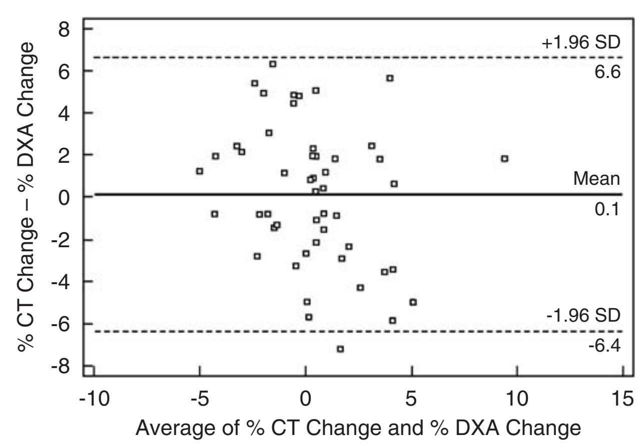

Figure 4 Bland–Altman plot of agreement between computed tomography (CT) and dual energy X-ray absorptiometry (DXA) methods for measuring percent muscle mass change in the untrained leg with strength training (ST) in older men and women ($n = 50$). CT and DXA methods were in agreement (mean difference = 0.13; 95% CI = −0.817 to 1.069). CI, confidence interval.

overestimation of muscle mass by DXA compared to CT. Finally, in three subjects DXA highly overestimated baseline and after training thigh SM mass (Figures 5 and 6).

Discussion

Our study demonstrates that DXA-measured changes in thigh SM mass with ST are correlated with changes in thigh SM mass as measured by multislice CT. However, changes in DXA-measured thigh SM mass are suspect due to the higher measurement error of DXA. Moreover, despite data suggesting agreement between the DXA and CT methods for measuring percent change of thigh muscle mass with ST, there is a lower correlation between the two methods for measuring thigh SM mass change. Finally, there was a very large absolute difference of $\sim$2 kg of thigh SM mass at baseline and after ST between the two methods, with an overestimation of thigh SM mass by DXA. Taken together, these data suggest that DXA may not be a valid method for measuring small changes in thigh SM with ST.

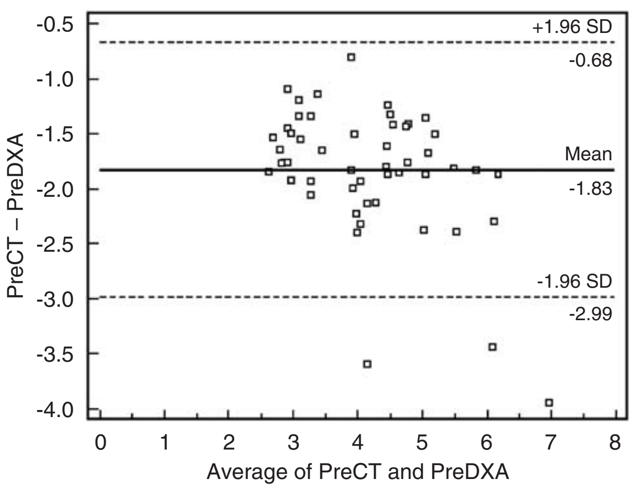

Figure 5 Bland–Altman plot of agreement between computed tomography (CT) and dual energy X-ray absorptiometry (DXA) methods for measuring baseline muscle mass in the trained leg in older men and women ($n=50$). CT and DXA methods were not in agreement (mean difference = −1.83 kg; 95% CI = −2.000 to −1.664). CI, confidence interval.

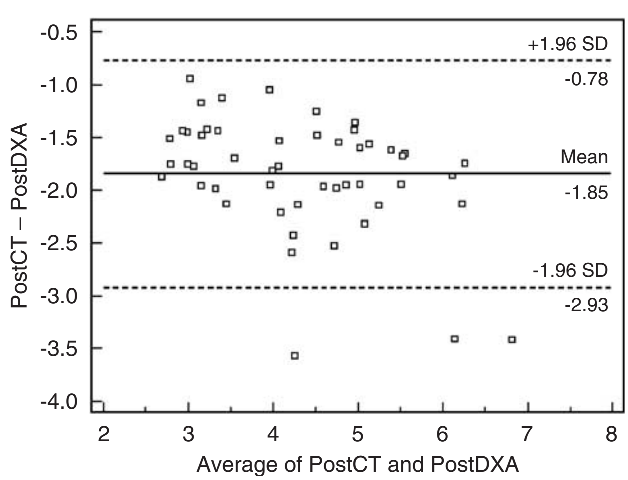

Figure 6 Bland–Altman plot of agreement between computed tomography (CT) and dual energy X-ray absorptiometry (DXA) methods for measuring after strength training (ST) muscle mass in the trained leg in older men and women ($n=50$). CT and DXA methods were not in agreement (mean difference = −1.85 kg; 95% CI = −2.007 to −1.695). CI, confidence interval.

It is unclear why there is a lower correlation between the DXA- and CT-derived changes in SM mass with ST than at baseline. A likely explanation for this difference is the error associated with DXA. Although DXA is considered to be a reliable method for the measurement of appendicular lean soft tissue, error associated with the DXA (Fuller *et al*., 1999) is caused by the assumption that all non-fat and non-bone FFM is SM mass. Additionally, a decrease in fatty infiltration may have occurred that could not be assessed by DXA, as DXA is not well suited to measure the change in this fat store. This could have lead to a systematic bias resulting in a lower estimate of muscle change when compared to CT. Previous investigations found that subfascial fat is more metabolically active than subcutaneous fat (Goodpaster *et al*., 2000b) and may be significantly affected by interventions such as exercise or weight loss (Goodpaster *et al*., 2000a). Finally, this difference could also be due to the ROI used. We attempted to set the upper and lower limits of the thigh at the same point for each method. However, it is possible that the accuracy of the DXA software may have resulted in a larger ROI than was used in CT scanning. Nevertheless, these possible sources of error are unlikely to explain fully the $\sim$2 kg overestimation of thigh SM mass by DXA at baseline and why seven of the subjects (14%) in the current investigation had an increase in thigh muscle mass as measured by CT, but had a decrease in thigh SM mass as measured by DXA.

The only other investigation that examined muscle mass changes from DXA and CT before and after ST in older adults did not compare the two methods (Nelson *et al*., 1996). That investigation reported no changes in aSM mass as measured by DXA. However, that study used only a mid-thigh CT slice to measure SM area changes with ST. In addition, the relatively small sample size used in that investigation may have limited the statistical power of that study to detect small changes in DXA-estimated SM mass. However, another investigation in young women compared leg DXA-estimated leg SM mass and mid-thigh CSA measured by MRI and found a correlation between the two measures with regard to change in lean tissue with ST (Nindl *et al*., 2000). That study however was limited by use of a cross-sectional scan of the mid-thigh, which has been shown not to be a valid predictor of overall aSM mass (Tracy *et al*., 2003).

The use of the untrained leg in the present study also adds a distinctive contribution to the current literature on this topic. Our data show that the untrained leg was a valid control because unlike strength, there was no cross-education effect on muscle mass with ST in the untrained leg as measured by either DXA or CT. These data confirm its value as a control for the normal drift in values due to variations in methodology, biology, season of the year, genetic differences between groups or differences in attention between experimental and control groups.

Nonetheless, there are some limitations to the current investigation. First, subjects were not homogeneous with respect to race. There were 15 African Americans and two Asian Americans in this cohort, with remainder of subjects being Caucasian. This could result in variability in the amount of thigh SM mass change detected, as there is evidence that the amount of SM lost and increases in fatty infiltration in SM varies between Caucasians and African Americans with aging (Visser *et al*., 2003; Song *et al*., 2004). Second, this ST protocol only targeted the knee extensors. This likely resulted in a lower absolute change in total thigh muscle area and increased the possibility that the measurement error played a more important role in the findings by reducing the percent muscle mass change observed. Third, validity of DXA measurements can be affected by hydration status (Tylavsky *et al*., 2003b). Normal water content of FFM is 72–74% with a s.d. of $\sim$3% (Lohman *et al*., 2000). Thus, an increase or decrease of $\sim$5% in hydration levels may lead to a systematic bias only to the extent of $\sim$1–2%, which falls within the range of s.d. of the DXA-measured changes. Hence, normal fluctuation in hydration level is not likely a major source of variation in DXA body composition estimates (Evans *et al*., 1999; Lohman *et al*., 2000). Moreover, our subjects were instructed to consume adequate amounts of water and repeat the same meal before testing.

This study highlights the limitations of using DXA to measure small changes in thigh SM mass with ST. Despite increases in thigh SM mass with ST as measured by DXA, the error associated with DXA calls into question the validity of these observed changes. Although DXA has certain advantages that warrant is used in large research studies, improvements to reduce its error are needed for the accurate calculation of thigh SM mass. Future studies should determine the specific underlying causes of the differences between the DXA and CT methods when estimating ST-induced changes in thigh SM mass. Finally, a model for correcting the DXA overestimation of thigh SM mass should be pursued, such as was performed using the older, pencil-beam DXA (Wang *et al*., 1999).

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